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RESEARCH ARTICLE

Preparation of chitosan/ alginate microcapsules by high-voltage electrostatic method

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Chitosan and sodium alginate have the opposite charges; they can become a gelatin by the electrostatic attraction, High-voltage electrostatic droplet generator method was used to prepare chitosan-sodium alginate microcapsule. Multi-layer chitosan-sodium alginate microcapsule was prepared through layer-by-layer self-assembly, and the morphology was investigated. In addition, the release property of ofloxacin in microcapsules was studied by UV-Vis microscopy under different conditions such as pH value, layer number, etc. The results showed that the prepared microcapsules have a smooth surface with average particle size about 100 μm. The result of controlled release indicated that the prepared microcapsules are pH-independent, and the rate of release decreased when the layer number increases.

Keywords chitosan, sodium alginate, electrospraying

1 Introduction

Microcapsule, which is one of the preferable systems used to physically envelope the sensitive ingredients in a protective material, can protect the sensitive ingredients from loss, adverse reaction, or against light [1] and has attracted much attention in biomedical fields such as cell culture, medicine, drug delivery, micro-reactor and catalysis.

Chitosan and alginate are natural cationic or anionic polysaccharides. They have been used a lot in the biomedical field because of their good biocompatibility, biodegradability, easiness to use and low price owing to their abundance in Nature [2–4]. The interaction between chitosan and alginate due to the amino and carboxyl groups in chitosan and alginate molecules have been systematically investigated [5]. Their complex was used as devices for the drug release [6,7]. The polymer fibers of alginate-based chitosan do well in promoting biologic responses of seeded chondrocytes to enhance cell attachment and proliferation [8].Among the chitosan microencapsulation techniques, several methods are employed for the synthesis such as solvent emulsification/ solvent evaporation [9,10], coacervation/ precipitation [11–13], spray-drying [14], and ionic gelation [15]. Generally, chemical cross-linkers and high speed stirring were used during the synthesis process. The chemical crosslinking agents are difficult to remove and would lead to undesirable toxic effects. And high speed stirring can interfere with the activity of the bioactive agents and the mechanical properties of the microcapsules [16].

Compared with these methods, electrospraying is a convenient way that an electric field is used to control the formation and deposition of polymers. At a critical voltage between a needle capillary end and a collector, the droplets will be produced by the electrostatic repulsion force of surface charges and dropped into the collector [17,18]. Due to the opposite charges of chitosan and alginate, multi-layer microcapsules can be fabricated through repeated deposition by the layer-by-layer (LbL) self-assembly technique [19–21]. And the thickness and composition can be controlled by the layer number and the polymer species [22,23].

The purpose of this paper was to find out a method to fabricate microcapsules at room temperature with simple operation, and the prepared microcapsules can encapsulate enzyme or protein to protect the activity. In this paper, (1) a high-voltage electrostatic droplet generator was used as a micro-encapsulation; (2) the morphology of the microcapsules was observed by the fluorescence microscopy (FM) and scanning electron microscope (SEM); (3) multi-layer microcapsules were fabricated by means of electrospraying using layer-by-layer self-assembly in solution; (4) the effects of pH value and the layer number on the release property of ofloxacin in microcapsules were investigated.

2 Experimental

2.1 Materials

Chitosan ($M_W = 1.2 \times 10^5$) with degree of deacetylation of 85% was purchased from Zhejiang Golden-Shell Biochemical Co., Ltd., China. Sodium alginate was obtained from Shanghai Sanfu Chemical Co., China. Ofloxacin ($C_{18}H_{20}FN_3O_4 \cdot \frac{1}{2}H_2O$) was supplied by Kunshan Double

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Crane Co., China. All the other reagents were purchased from Beijing Chemical Reagent Company and used without further purification.

2.2 Electrospraying

2.2.1 Electrospraying of sodium alginate solution with chitosan reception

Transparent solution of sodium alginate (2 wt%) was made by dissolving sodium alginate in 0.9 wt% sodium chloride with stirring for 1 h at room temperature. The collect solution was prepared by dissolving chitosan (1 wt%) and calcium chloride in 1 wt% formic acid solution with stirring for 0.5 h at room temperature. The solution of sodium alginate was transferred to a 5 mL medical plastic syringe fitted with a stainless steel needle and set up in the electrospraying apparatus (BMEI Co., Beijing, China). A positive high-voltage supply was used to maintain the voltage. To investigate the effects of the preparation conditions on the diameter of chitosan/sodium alginate microcapsules, an aluminum foil was grounded and located 10 cm apart from the capillary tip. The inner diameters of the steel needles were from 5 to 6 mm. The working voltage was controlled within 0–15 kV, and the feed rate of electrospinning solution was controlled at from 0.01 to 0.2 $mm \cdot s^{-1}$.

2.2.2 Preparation of multi-layer microcapsules

Chitosan and sodium alginate as polysaccharides were directly deposited on the microcapsules made by electrospraying using LbL self-assembly in solution. The chitosan encapsulation on the sodium alginate droplet was defined as the first layer. The excess chitosan was removed by two refining circles of centrifugation (5000 r/min, 5 min)/washing/re-disperse in water. The second layer was deposited by dispersing the microcapsules into the sodium alginate solution (2 wt%). The mixture was incubated for 15 min with gentle shaking. And the excess sodium alginate was removed in the same way. The following chitosan layer was deposited in the same procedure with the chitosan solution (1%). Alternate sodium alginate and chitosan layers were deposited successively in this identical way and microcapsule walls made of chitosan / sodium alginate multi-layer microcapsules were achieved.

2.3 Characterization

2.3.1 Analysis of electron microscopy (FM and SEM)

The structure of the microcapsules was observed with Fluorescence Microscopy (IX81, Olympus Company in Japan, using UV light, green light, and blue light respectively). The microcapsule samples were dried to dehydration at the room temperature, then sputter-coated with Au and characterized with a scanning electron microscope (Hitachi S-450, Tamura, Japan). The multi-layer chitosan/sodium alginate microcapsules were examined with SEM in the same way.

2.3.2 *In vitro* release

Ofloxacin was chosen as a model medicine to investigate the drug release property of the microcapsules. Ofloxacin was dissolved in the solution of sodium alginate (2 wt%) before electrospraying. Multi-layer microcapsules were prepared in the same way described above. The release properties were researched in various pH solutions (pH 4.84, pH 7.0 and pH 9.44). The release rate of the ofloxacin from the microcapsules was monitored with UV absorbance.

The microcapsules were first punched into 80 mL of pH 4.84 acetic acid-sodium acetate buffer solution (HAc-NaAc) at 37°C and 100 r/min in a thermostatically shaking incubator (HZ-9610K, Taicang instrument Co., Taicang, China). 2 mL of the samples were taken from the buffer solution at stated time intervals. The amount of ofloxacin released was determined by a UV-visible spectrophotometer U-3010 (Hitachi, Tamura, Japan) at the wavelength of 295 nm.

The results were presented in terms of cumulative release as a function of release time. The cumulative amount of release was calculated according to Eq. (1):

$$\text{Cumulative amount of release}(\%) = (M_t/M_\infty) \times 100\%, \quad (1)$$

where M_t was the amount of ofloxacin released at time t, and the amount of ofloxacin added to electrospraying solution was regarded as M_∞ in this paper. All samples were tested three times.

The sustained release behaviors in phosphate buffer solution (KH_2PO_4, pH 7.0) and natrium boricum buffer solution (pH 9.44) were studied according to the same procedure.

The multi-layer chitosan / sodium alginate microcapsules of 5 walls and 9 walls were determinate in the same way to compare the release rate of the ofloxacin at different layers.

3 Results and discussion

3.1 Effects of the preparation conditions on the diameter of chitosan/sodium alginate microcapsules

We researched the effects of the preparation conditions on the

diameter of chitosan/sodium alginate microcapsules. While one of the factors was varied, the other factors were kept the same. These factors included working voltage, needle inner diameter, and feed rate. Fig. 1 (a) showed the effect of the working voltage on the diameter of the microcapsules. The diameter of the microcapsules decreased from 1733 to 243 μm as the working voltage increased from 0 to 15 kV. The inner diameters of the needles we chose were 6 mm and 5 mm, respectively. And the effect of the inner diameter of the needle was studied (Fig. 1 (b)). The diameter of the microcapsules increased from 1733 to 2033 μm while the inner diameter of the needles were increased. As seen in the Fig. 1 (c), the effect of the feed rate on the diameter of microcapsules was investigated. The diameter of the microcapsules decreased from 243 to 214 μm as the feed rate decreased from 0.2 to 0.05 mm·s^{-1}. But the diameter of the microcapsules was increased to 252 μm when the feed rate was 0.01 mm·s^{-1}.

3.2 Morphology of microcapsule

The fluorescence microscope pictures of microcapsules were shown in Fig. 2 (200 μm in diameter, exposure time was 250 ms). As shown in Fig. 2, the microcapsules were particles with uniform diameter and smooth surface.

The morphology of the microcapsules was shown in Fig. 3. Figs. 3 (a) and (b) were the SEM micrograph which showed that microcapsules were dehydrated and their surfaces presented corrugation. From Fig. 3(c) we could distinctly see the multi-layer structure of microcapsules.

3.3 *In vitro* release

The drug release behavior of single and multi-layer microcapsules loaded with ofloxacin in different pH buffer solutions was investigated.

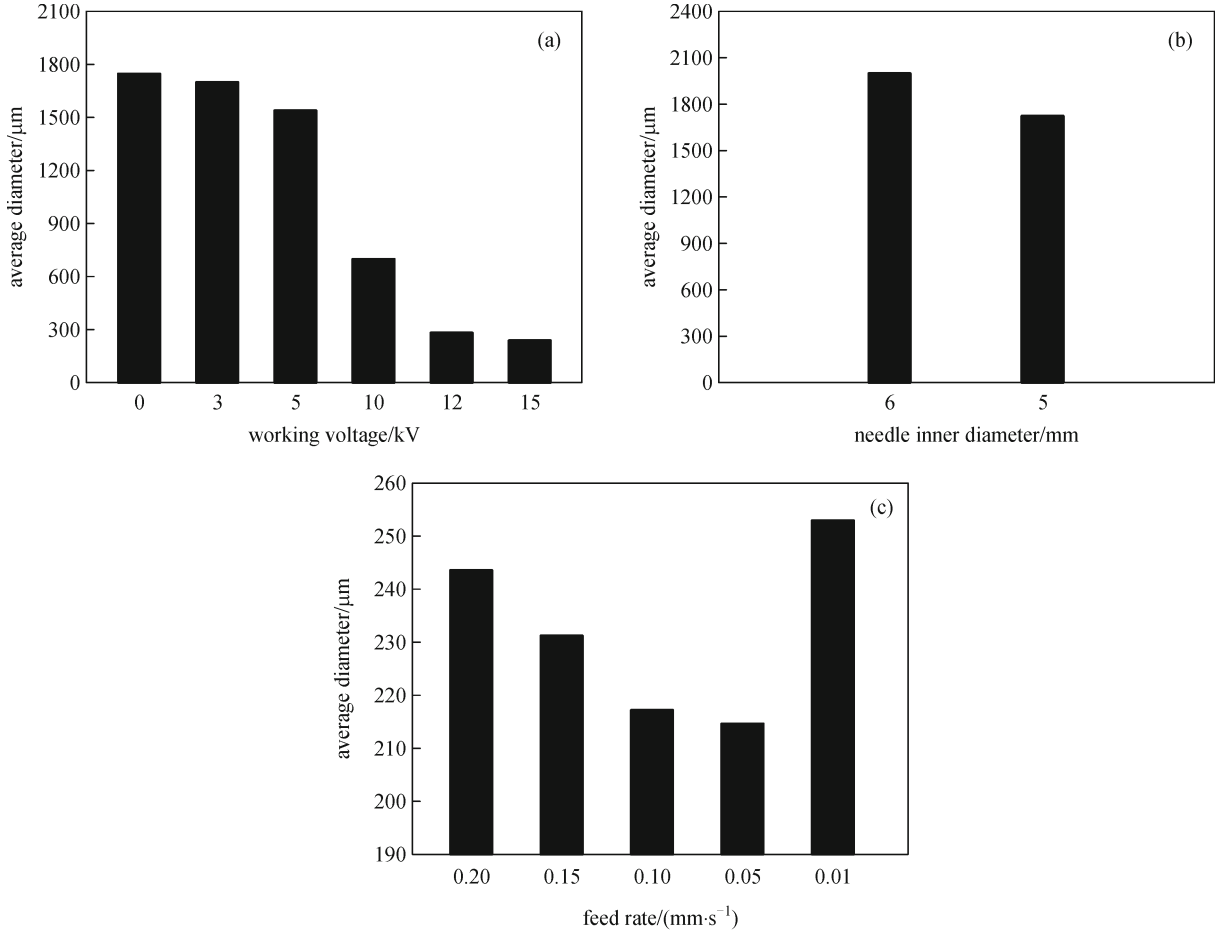

Figure 1 Effects of the preparation conditions on the diameter of microcapsules. (a) working voltage (needle inner diameter 5 mm, feed rate 0.2 mm·s^{-1}); (b) inner diameter of needle (working voltage 0 kV, feed rate 0.2 mm s^{-1}); (c) feed rate (working voltage 15 kV, needle inner diameter 5 mm).

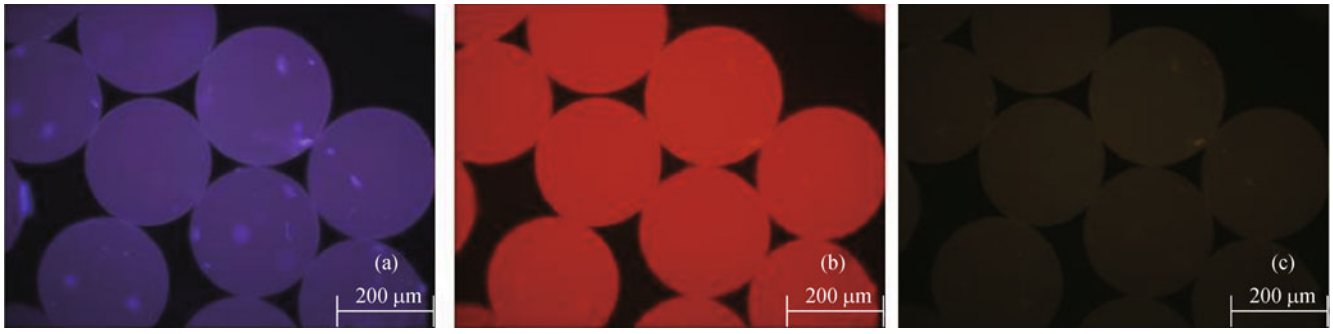

Figure 2 Fluorescence microscope pictures of microcapsules with the use of (a) UV light, (b) green light, (c) blue light

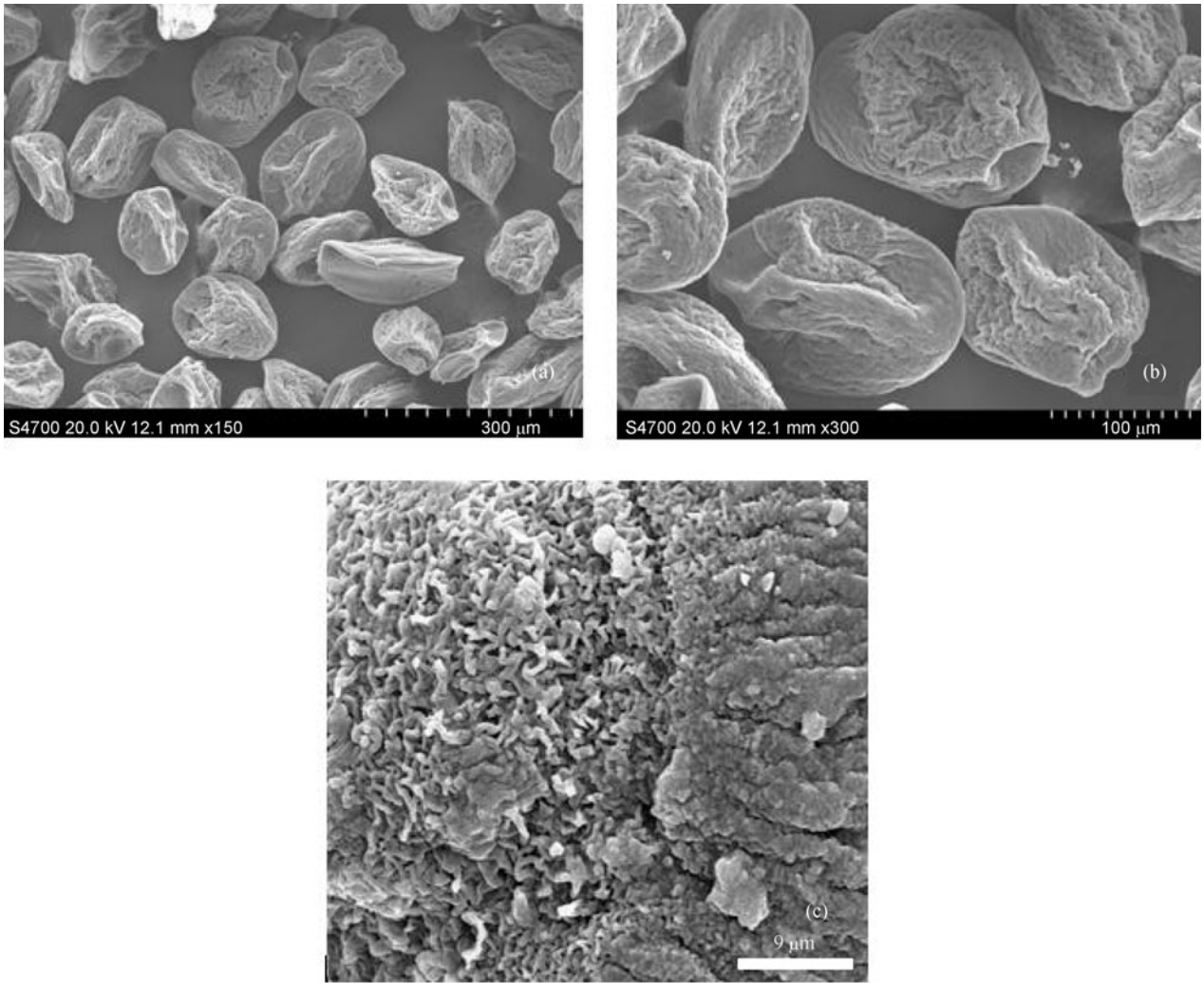

Figure 3 SEM images of the microcapsules: (a) dehydrated microcapsules; (b) magnification of (a); (c) multi layers microcapsule (five layers)

3.3.1 Effect of pH value of the buffer solutions

Chitosan and alginate are natural cationic or anionic polysaccharides, so they were sensitive to pH values of aqueous solution. Commonly, drug release of chitosan hydrogel was sensitive to acid environment, and drug release

of sodium alginate was sensitive to alkaline environment. However, the microcapsules prepared by chitosan and alginate did not show pH sensitivity. Fig. 4 illustrated the release property of the chitosan/sodium alginate microcapsules in buffer solutions with different pH values (4.84, 7.0, and 9.44 in pH). In the three different buffer solutions, the ofloxacin release rate and final ofloxacin release percentage were similar. They all released fast in the first 40 min and got the inflexion. It was possibly because that the amino group in the chitosan and carboxyl group in the alginate were complex by electrostatic attraction during the formation of gelatin, so the chitosan/sodium alginate microcapsules were independent of the pH value of the buffer solutions.

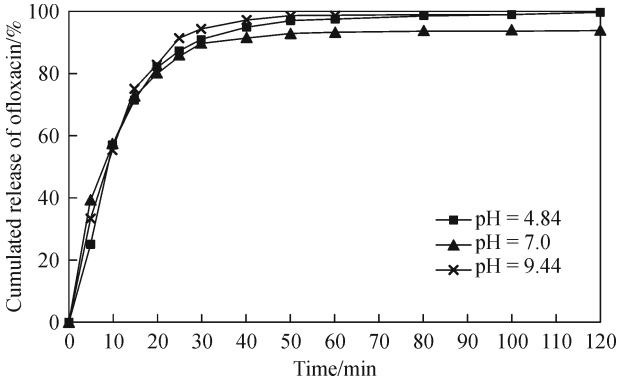

Figure 4 Cumulated release of ofloxacin in buffer solutions with pH 4.84, 7.0 and 9.44 (single layer of microcapsules)

3.3.2 Effect of layers of the multi-layer microcapsules

Release curves of ofloxacin from microcapsules with one layer, five layers and nine layers were plotted in Fig. 5, and compared on the basis of the same ofloxacin loadings. The release rate of ofloxacin from nine layers microcapsules was slightly alleviated during the initial release and showed sustainability compared to that of the single layer. Although the drug release effect of multi-layer microcapsules was obviously not ideal, the study at least gave us a hint that we could change the layers of microcapsules and the methods of the drug load to improve the drug release effect. In general, more layers and smaller amount drug loading could produce better effect of drug controlled release.

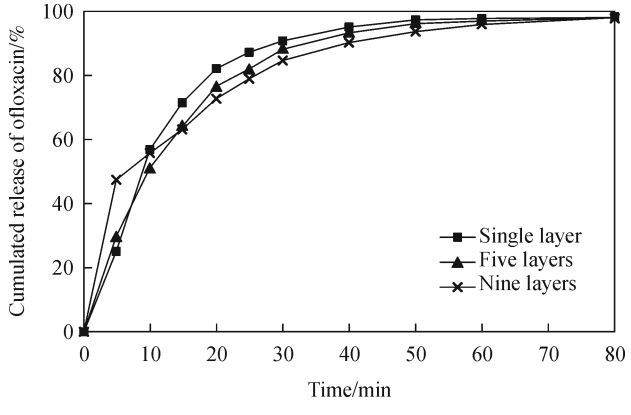

Figure 5 Cumulated release of ofloxacin from microcapsules with different layers in solution with pH 4.84

4 Conclusions

Electrospraying is a versatile method for incorporation of bioactive macromolecules such as proteins, growth factors and DNA, and they could be released steadily from the micro-nano capsules produced. This method has the advantages of facileness, high loading efficiency, mild preparation condition and can avoid inactivation of the biologic active substances in the process of preparation of the microcapsules. In this paper, the prepared chitosan/sodium alginate multi-layer microcapsules by electrospray technology is featured by particle size control, better uniformity of particle size, and the ability to load drugs and improve the drug release effect through regulating the layers of the microcapsules. This study provided only a basis for further designing and optimization of core-shell nanostructure and a potential toward final achievement of desired release purpose of bioactive agents for gene therapy and tissue engineering.

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References

1. Vinetsky, Y.; Magdassi, S., Microencapsulations in Cosmetics. In: Magdassi S, Touitou E(eds.). Novel Cosmetic Delivery Systems. New York: Marcel Dekker, Inc., 1998, 295–313
2. Huguet, M. L.; Groboillot, A.; Neufeld, R. J.; Poncelet, D.; Dellacherie, E., *J. Appl. Polym. Sci*. **1994**, 51, 1427–1432
3. Gombotz, W. R.; Wee, S. F., *Adv. Drug Deliv. Rev.* **1998**, *31*, 267–285
4. Ramadas, M.; Paul, W.; Dileep, K. J., Ramadas, M.; Anitha, Y.; Sharma, C. P., *J. Microcapsulation* **2000**, *17*, 405–411
5. Becherán-Marón, L.; Peniche, C.; Argüelles-Monal, W., *Int. J. Biol. Macromol.* **2004**, *34*, 127–133
6. Murata, Y.; Kontani, Y.; Ohmae, H.; Kawashima, S., *Eur. J. Pharm. Biopharm.* **2002**, *53*, 249–251
7. Ribeiro, A. J.; Silva, C.; Ferreira, D.; Veiga, F., *Eur. J. Pharm. Sci.* **2005**, *25*, 31–40
8. Iwasaki, N.; Yamane, S. T.; Majima, T.; Kasahara, Y.; Minami, A.; Harada, K.; Nonaka, S.; Maekawa, N.; Tamura, H.; Tokura, S.; Shiono, M.; Monde, K.; Nishimura, S., *Biomacromolecules* **2004**, *5*, 828–833

9. Denkbaş, E. B.; Seyyal, M.; Pişkin, E., *J Microencapsul* **1999**, *16*, 741–749
10. Jameela, S. R.; Kumary, T. V.; Lal, A. V.; Jayakrishnan, A., *J. Control. Release* **1998**, *52*, 17–24
11. Ozbaş-Turan, S.; Akbuğa, J.; Aral, C., *J Pharm Sci* **2002**, *91*, 1245–1251
12. Mao, H. Q.; Roy, K.; Troung-Le, V. L.; Janes, K. A.; Lin, K. Y.; Wang, Y.; August, J. T.; Leong, K. W., *J. Control. Release* **2001**, *70*, 399–421
13. He, P.; Davis, S. S.; Illum, L., *Int. J. Pharm.* **1999**, *187*, 53–65
14. Shi, X. Y.; Tan, T. W., *Biomaterials* **2002**, *23*, 4469–4473
15. Pan, Y.; Li, Y. J.; Zhao, H. Y.; Zheng, J. M.; Xu, H.; Wei, G.; Hao, J. S.; Cui, F. D., *Int. J. Pharm.* **2002**, *249*, 139–147
16. Ko, J. A.; Park, H. J.; Hwang, S. J.; Park, J. B.; Lee, J. S., *Int. J. Pharm.* **2002**, *249*, 165–174
17. Doshi, J.; Reneker, D. H., *J. Electrost.* **1995**, *35*, 151–160
18. Reneker, D. H.; Chun, I., *Nanotechnology* **1996**, *7*, 216–223
19. Sukhorukov, G.; Dähne, L.; Hartmann, J.; Donath, E.; Möhwald, H., *Adv. Mater. (Deerfield Beach Fla.)* **2000**, *12*, 112–115
20. Gao, C. Y.; Donath, E.; Möhwald, H.; Shen, J., *Angew. Chem. Int. Ed. Engl.* **2002**, *41*, 3789–3793
21. Dähne, L.; Leporatti, S.; Donath, E.; Möhwald, H., *J. Am. Chem. Soc.* **2001**, *123*, 5431–5436
22. Caruso, F.; Caruso, R. A.; Möhwald, H., *Science* **1998**, *282*, 1111–1114
23. Okubo, T.; Suda, M., *Colloid Polym. Sci.* **2002**, *280*, 533–538